

An *in Vivo* Evaluation of the Immunosuppressive Action of Bleomycin¹

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SUMMARY

The new antineoplastic antibiotic, bleomycin, was evaluated in a non-*H-2* allograft system (DBA/2 → BALB/c) for immunosuppressive action. At high doses, the drug significantly altered graft rejection times. The high mortality and severe debility at these doses, however, suggest that the prolonged allograft survival times were not mediated by specific immunosuppression.

INTRODUCTION

The use of cancer chemotherapeutic agents has always been hampered by the adverse side effects associated with these drugs. In general, these agents do not have a preferential effect on the tumor cells but, rather, affect rapidly growing cells in a nonspecific manner. The most frequent and generally the most severe complication involves the bone marrow, with the production of subsequent leukopenia and thrombocytopenia (6). The resulting immunosuppression is often self-defeating from several standpoints. First, immunosuppressed individuals are more susceptible to fulminating secondary infections. Second, evidence generated by tumor immunology research in the last 10 years indicates that immunity plays a significant role in preventing or curtailing certain neoplasms. Recently, a new antineoplastic antibiotic has been introduced which has a number of desirable attributes. Bleomycin, a basic peptide produced by *Streptomyces verticillus*, has been demonstrated to be highly effective against numerous squamous cell cancers as well as a variety of other tumor types. Recent clinical trials in Europe and the United States have confirmed the initial Japanese studies as to the effectiveness of the drug (2, 3, 8, 9). Of particular interest has been the apparent lack of hematopoietic or immunological depression associated with the use of this drug. The inability of bleomycin to affect primary antibody responses in mice has recently been reported (5). This study confirms the nonimmunosuppressive properties of the drug as reported by clinicians. In a recent report, however, Mathé (2) has cited unpublished results of immunosuppression by bleomycin in the Jerne plaque test. It was felt, therefore, that the question of the immunosuppressive activity of bleomycin, especially as regards cellular immunity, was of sufficient importance to warrant an investigation *in vivo*. This study was done to determine

allograft rejection times in mice treated with various concentrations of the drug.

MATERIALS AND METHODS

Animals. Inbred BALB/c and DBA/2J mice were obtained from The Jackson Laboratories, Bar Harbor, Maine. These strains were specifically chosen because they share the same strong histocompatibility allele (*H-2^d*), but differ by alleles at multiple non-*H-2* loci. The animals were housed 6 per stainless steel cage and were given Purina laboratory chow and water *ad libitum*.

Skin Grafting. The grafting technique has been reported previously (1). Split-thickness skin grafts (13 mm in diameter) were prepared from female DBA/2J donors and were placed on female BALB/c recipients. Tapes were removed from the animals 7 days after grafting, and the grafts were examined daily thereafter.

Bleomycin. Bleomycin was obtained from Dr. Richard Bornstein at the American Oncologic Hospital, Philadelphia, Pa. Extrapolation of the dosage from man to mouse was as described by Freireich *et al.* (4). Based on the average total human dose of 4 mg/kg, the calculated equivalent dose for an average 20-g mouse was 1.056 mg (52 mg/kg). The drug was constituted in sterile HBSS² so that 1 ml of solution contained the calculated dose. Animals were given i.p. injections of 0.25 ml of solution on the day before grafting and on Days 1, 3, and 5 after grafting. Earlier toxicity trials showed the LD₅₀ of bleomycin in these BALB/c mice to be 2.112 mg total dose (105 mg/kg). Each animal (in experimental groups of 12) received a total dose of 0.528, 1.056, or 2.112 mg. These quantities correspond respectively to one-half, full, and double the calculated human equivalency dose. A control group of 12 animals received HBSS only.

RESULTS

Of the groups receiving injections of bleomycin, deaths occurred only in the high-dose group receiving 2.112 mg. After the 1st injection, these animals became lethargic, assumed a hunched position, had rough hair coats, and in general emulated all the characteristics of sick mice. Fifty % of the animals in this group died. However, in earlier toxicity trials, the same total dose given in 0.264-mg daily increments for 8

¹ This study was aided in part by Grant IC-46 from The American Cancer Society.

Received July 12, 1971; accepted June 7, 1972.

² The abbreviation used is: HBSS, Hanks' balanced salt solution.

Table 1
Mean survival times of allografts from female DBA/2J donors on
BALB/c female mice given injections of HBSS
or various dosages of bleomycin

Group treatment ^a	No. of mice surviving/total no. given injections	Graft survival (days; mean \pm S.E.)
HBSS	12/12	10.16 \pm 0.97
Bleomycin, 0.528 mg	12/12	12.16 \pm 0.41
Bleomycin, 1.056 mg	12/12	11.45 \pm 0.64
Bleomycin, 2.112 mg	6/12	15.33 \pm 0.33 ^b

^a Quantities are total doses given as 4 separate i.p. injections

^b Significantly different from all other groups ($p < 0.002$).

days failed to kill a single animal or produce notable changes in appearance.

The mean graft survival times for the animals in various groups are shown in Table 1. Only the animals receiving the 2.112-mg total dose had a significantly longer allograft survival time when compared with the other dosage groups or with the control animals given HBSS. By Mann-Whitney U test examination (7), this difference was significantly different from all other groups with $p < 0.002$.

DISCUSSION

It has been reported clinically that bleomycin, an active antineoplastic antibiotic, is not toxic to bone marrow, nor does it depress peripheral leukocyte values (2, 8). Similarly, in experimental animals, the drug has failed to effect secondary antibody production even at doses comparable to those used in man (5). Mathé (2) has recently suggested that bleomycin has an immunosuppressive property, when examined *in vitro* by the Jerne plaque technique. This technique is an extremely sensitive test aimed primarily at the detection of humoral immunity. In order to evaluate the effects of bleomycin on cellular immunity, this study was done.

At total dosages comparable to those used in man, bleomycin has no detectable effect on skin graft survival in this weak histoincompatible system (Table 1; group given 1.056 mg). Dosages that correspond to twice the total human dose (2.112 mg) killed 50% of the animals in that group and resulted in increased allograft survival time. This increase, while statistically significant, is not considered to be a true index of immunosuppression in this system. First, the dosage necessary to produce the effect is extremely high. Second, the effect was mild as judged by the length of survival (approximately 15 days). In systems in which recognized immunosuppression is achieved by such treatments as

thymectomy and antithymocyte serum, allograft survival with the same mouse strain combinations averages greater than 38 days (1). The severe debilitation which resulted from the high-dose injections further suggests that the findings were not due to specific immunosuppression. That this debility was produced by frequent injections of high doses is exemplified by the fact that in earlier toxicity trials with BALB/c mice the same total dose (2.112 mg), when given in equal 0.264-mg increments over 8 days produced no debility or deaths. Severe stress will temporarily impair immunological capabilities, especially as regards cellular immunity. It is conceivable, therefore, that the inability of these mice to reject the grafts was due to debility and, once the injections were stopped, rapid recovery resulted in relatively fast rejection of the allografts.

In conclusion, bleomycin does not appear to be a potent immunosuppressive agent in mice even when administered in doses that far exceed those used in man.

ACKNOWLEDGMENTS

The skilled technical assistance of Mrs. Nancy McClintock is acknowledged.

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